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Seasonal and diurnal variations in DIC, NO_3^- and TOC concentrations in spring-pond ecosystems under different land-uses at the Shawan Karst Test Site, SW China: Carbon limitation of aquatic photosynthesis

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ABSTRACT

Human activities have altered terrestrial carbon (C) and nitrogen (N) dynamics via changes to land cover and use such as deforestation, agriculture, application of fertilizers, etc. and have influenced the patterns of organic C input and eutrophication in downstream freshwater ecosystems. Biogeochemical cycling of C and N and the related organic carbon (OC) production may display correlated diurnal and seasonal variations due to photosynthesis and respiration in these ecosystems, whose underlying mechanisms still need to be resolved. In this study, we document the diurnal and seasonal variations measured in DIC (dissolved inorganic carbon), NO_3^-, TOC (total organic carbon) and other related hydrochemical parameters (pH and DO-dissolved oxygen) in five artificial spring-pond ecosystems with differing land-uses in tanks draining into springs and corresponding ponds, constructed at the Shawan Karst Test Site, SW China. It was found that diurnal changes in DIC, NO_3^- and TOC in all ponds were dominated by aquatic ecosystem metabolism (i.e., photosynthesis and respiration), as evidenced by the pertinent variations in DO and pH. Daily DIC and NO_3^- uptake and OC production were higher in October (growing period) and lower in January (dormant period), indicating seasonal differences in assimilation that were determined by both changes in weather (temperature and light) and nutrient inputs. Under conditions of bare rock or bare soil, there was very low DIC and NO_3^- additions to the spring-pond ecosystems, resulting in lower OC productivity in the ponds. Cropped land yielded higher DIC and NO_3^- to the pond, due to growth of corn and use of fertilizers that enhanced OC production. Highest productivity and densest vegetation cover on tanks with grassland or shrubs (with higher N retention in soils) resulted in higher DIC but limited NO_3^- addition to the ecosystems downstream. The highest DIC concentration (in the grassland) resulted in maximum OC production in the pond. These results indicate that OC production in the ponds with elevated pH was limited by DIC fertilization. In general, the supply of DIC is not considered to limit aquatic primary productivity because its concentration exceeds that of other plant macronutrients such as NO_3 and PO_4^{3-} by two or three orders of magnitude. Therefore, the carbon limitation detected here may indicate that photoautotrophs in karst dominated aquatic terrains (dominated by *Charophyta* and *Spirogyra*) cannot use the total DIC for photosynthesis but only the dissolved CO_2, which comprises $< 1\%$ of total DIC at pH > 8.2 that is characteristic in these environments. This may have implications for control of eutrophication in such alkaline aquatic ecosystems, i.e., rates of eutrophication in freshwater ecosystems may be regulated not only by N and/or P but also by C. It is also projected that there will be an increase in OC sequestration with the current land-use and global climate change-driven increases in DIC, due to carbon limitation of aquatic primary production.

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1. Introduction

Imbalances in global carbon (C) and nitrogen (N) budgets have become important environmental issues because of their inherent connection with contemporary climate change (Melnikov and O'Neill, 2006; Schlesinger, 2009). Coupled carbonate weathering-derived carbon sequestration in inland water ecosystems by the so-called terrestrial biological carbon pump effect (TBCP) has been found in several studies (Chen et al., 2017; Jiang et al., 2013; Liu et al., 2010; Liu et al., 2015; Liu et al., 2018; Yang et al., 2015). The higher dissolved organic carbon (DIC; half of which from soil/atmosphere) from carbonate weathering can fuel the growth of aquatic plants and thus enhance the uptake of atmospheric carbon (Liu et al., 2018). Contemporary human activities such as agricultural land-use and land-cover changes (LUCC) have resulted in increased carbon and nitrogen discharge from soils into aquatic ecosystems, and thus significantly increased the DIC and nitrate concentrations in surface and groundwater systems (Mulholland., 2008; Raymond et al., 2008; Taylor and Townsend, 2010; Zeng et al., 2017).

For a long period CO_2 increase has been thought to have only minor influence on primary productivity in freshwater ecosystems because of the common assumption that CO_2 concentrations in freshwater are high enough for phytoplankton growth (Visser et al., 2016). However, the actual concentration of dissolved CO_2 is quite low and CO_2 uptake by flourishing submerged aquatic plants can turn the water from supersaturated to highly undersaturated to atmospheric CO_2 (Balmer et al., 2011). Phytoplankton photosynthesis draws down the concentration of dissolved CO_2, leading to the water becoming CO_2-undersaturated, increasing the pH and changing principal molecular hosts of dissolved inorganic carbon (DIC) (Talling, 1976; Verschoor et al., 2013). As shown in Fig. 1, a high pH environment helps shift the inorganic carbon dominated by $CO_{2(aq)}$ towards a bicarbonate and/or carbonate ion dominated system (Schulte et al., 2011). This severely reduces the concentration of dissolved $CO_{2(aq)}$ in water, which is only 1% of DIC at pH 8.2 (Riebesell et al., 1993). $CO_{2(aq)}$ is the favored substrate for aquatic plant photosynthesis. Therefore, elevated pH may be associated with accessible carbon limitation for phytoplankton production (Van Dam et al., 2018; Verspagen et al., 2014). To relieve this limitation, some phytoplankton have developed an ability to use bicarbonate and carbonate by CO_2-concentrating mechanisms (CCMs), allowing them to access additional inorganic carbon pools when $CO_{2(aq)}$ becomes depleted from increase in pH (Mangan et al., 2016; Morales-Williams et al., 2017; Price et al., 2008; Price, 2011; Van Dam et al., 2018).

On the other hand, as a fundamental nutrient, N availability may also constrain the algal growth, carbon uptake and primary production of aquatic ecosystems (Elser et al., 2007). N alteration caused by different land-use activities can impact the nutrient uptake rate and N dynamics (Hall et al., 2009), causing eutrophication problems in aquatic ecosystems (Abell et al., 2010; Conley et al., 2009; Howarth and Marino, 2006; Jarvie et al., 2018). The cycling of C and N in freshwater has been shown to be closely related, with coupled control of organic carbon (OC) concentrations through aquatic biological processes of assimilation or denitrification (Seitzinger et al., 2006; Gruber and Galloway, 2008; Taylor and Townsend, 2010; Trimmer et al., 2012). Further, a growing body of evidence has shown that C, N and OC production in freshwater ecosystems display similar patterns of change that are most likely due to aquatic ecosystem metabolism (Clarke et al., 2002; Harrison et al., 2005; Heffernan and Cohen, 2010; Jiang et al., 2013; Parker et al., 2007; Parker et al., 2010; Poulson and Sullivan, 2010; Spencer et al., 2007). Nutrient load such as N input has long been thought to be the most significant limiting factor for organic matter production in aquatic ecosystems, compared to the effect of C, which seems to have less influence (Hecky and Kilham, 1988; Visser et al., 2016). However, laboratory experiments and models have demonstrated that rising CO_2 concentrations may result in phytoplankton blooms in both marine (Chen and Durbin, 1994; Riebesell et al., 1993) and freshwater ecosystems (Hein and Sand-Jensen, 1997; Verschoor et al., 2013; Verspagen et al., 2014; Young and Gobler, 2017) by reducing the C limitations on primary production. Moreover, in freshwater ecosystems, elevated CO_2 uptake can increase the carbon:nutrient stoichiometry in aquatic biomass (Verspagen et al., 2014). In addition, primary producers still have the ability to photosynthesize when sufficient light and DIC are available, even under severe nutrient-limited conditions (Verschoor et al., 2013).

It is generally difficult to distinguish and estimate the impacts of specific land-use activities on C and N inputs and their subsequent effects on aquatic C and N cycles in natural conditions where, different land-uses persist within a given catchment. Studies in single-use catchments can clearly help us to determine the C and N dynamics and their relationships with aquatic primary production. This is best accomplished under experimental control of land-use types. Therefore, we have investigated the spring-fed pond ecosystems derived from five different experimental land-uses at the Shawan Karst Test Site (SW China), measuring the range of DIC and NO_3^- concentrations by each individual spring to its hydrologically connected artificial pond (Chen et al., 2017; Zeng et al., 2017). The different types of land-use in the experiment (bare rock, bare soil, cropland, grassland, and shrubland) displayed large DIC and NO_3^- gradients, and the different nutrient input scenarios helped us to determine the role of C vs N limitation in aquatic ecosystem photosynthesis and its potential influence on eutrophication control and carbon cycling in karst surface waters with high pH and DIC. Though the biological carbon pump effect has been found to be related to the DIC fertilization by previous authors (Chen et al., 2017; Liu et al., 2015; Yang et al., 2015; Yang et al., 2016), the underlying mechanisms still need to be resolved, especially the roles of principal molecular hosts of DIC, which may have implications in controlling eutrophication and the carbon cycle in such aquatic ecosystems.

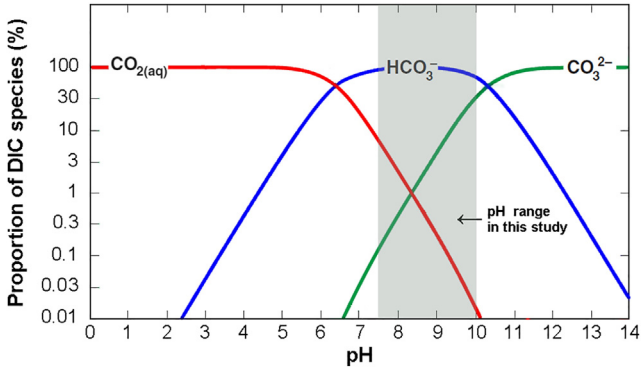

Fig. 1. Proportions of dissolved inorganic carbon (DIC) species ($CO_{2(aq)}$, HCO_3^- and CO_3^{2-}) as a function of pH, for freshwater at a temperature of 25 °C (modified after Schulte et al., 2011).

2. Site description

Our test site is located in the Puding Comprehensive Karst Research and Experimental Station, Puding County, Guizhou Province, Southwest China (Fig. 2(a)). The site has a humid sub-tropical monsoon climate with annual mean air temperature of 15.1 °C. Mean annual precipitation is 1315 mm, 80% of which falls in the wet season from May to October. In January 2014, five concrete tanks, LU1 – LU5, were built at the experimental station, each of them simulating a single land-use catchment, as shown in Fig. 2(b–d). Each tank is 20 m long, 5 m wide, and 3 m deep, coated with epoxy resin (to avoid the influence of possible concrete erosion on the tank hydrochemistry), filled with 2 m dolomitic limestone rubble in the lower part and topped with 0.5 m soil. Tank LU1 was left with rubble only, intended to simulate the scenario with no soil and plants on the land but only bare carbonate rocks (rocky desertification). Tank LU2 simulates land with only bare soil and the carbonate rubble beneath it. Tank LU3 was planted with corn to

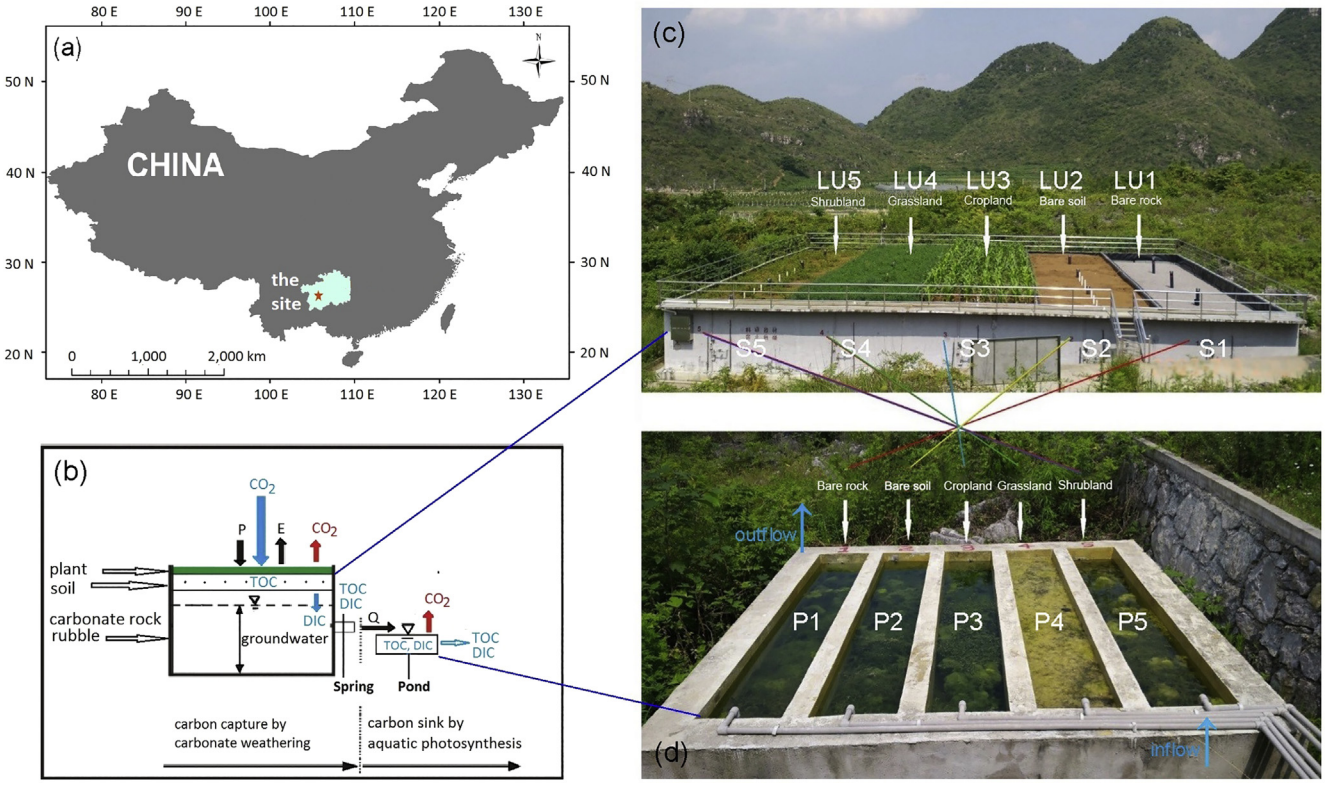

Fig. 2. (a) Site location; (b) general structure and carbon footprint of the spring-pond system; (c) the five land-use simulation tanks, (d) overview of the model spring-fed ponds. Five types of land-use feeding the connected ponds with flourishing submerged plants are bare rock, bare soil, cropland, grassland and shrubland. P: precipitation; E: evapotranspiration; Q: spring discharge; DIC: dissolved inorganic carbon; TOC: total organic carbon.

Table 1
Land use types of five tanks and their connected spring-pond systems.

Tank number	LU1	LU2	LU3	LU4	LU5
Simulated land-use type	bare rock	bare soil	cropland	grassland	shrubland
Spring-pond system	S1-P1	S2-P2	S3-P3	S4-P4	S5-P5

simulate intensive cultivation with application of nitrogen fertilizers. Alfalfa and Roxburgh rose were sown in the soils of Tanks LU4 and LU5 to simulate grass and shrub land-uses, respectively. A lateral drainage hole in each tank simulated a natural karst spring (S1 – S5) which fed an artificial pond (P1 - P5) that was 3 m long, 0.5 m wide, and 0.5 m deep (Fig. 2(d)), to simulate a lake or a reservoir. Equal amounts of locally dominant submerged plants, including *Spirogyra*, *Hornwort* and *Charophyta* were transplanted into each spring-fed pond in January 2014. Each tank and its connected pond system (Fig. 2(c–d)) thus simulate a single land-use catchment (Table 1) with precisely known, identical dimensions and all share the same climatic and geological conditions (Chen et al., 2017; Zeng et al., 2017).

3. Methods

3.1. Field monitoring of seasonal and diurnal hydrochemistry

Four sampling surveys (19–21 July and 24–26 October 2015, 23–25 January and 26–28 April 2016), were conducted to examine seasonal and diurnal biological controls on each spring-pond chemistry at the same times. The hydrochemistry was monitored automatically during sunny weather with steady water flow conditions for 48 h, to evaluate the diurnal variation of spring waters, S1-S5, and pond waters, P1-P5. The monitoring period in each season thus was two full diurnal cycles. Five Manta Multi-parameter Data Logger 2.0 and five WTW Technology Multiline 350i were programmed to record at 15-min intervals for each spring vent and pond outlet, respectively. The measured parameters were water temperature (T), pH, electrical conductivity (EC, 25 °C), and dissolved oxygen (DO). The instruments were calibrated prior to deployment using pH (4, 7 and 10), EC (1412 μs cm^{-1}), and DO (0% and 100%) standards. Resolution of pH, T, DO and EC were 0.01, 0.01 °C, 0.01 mg L^{-1} and 0.01 μs cm^{-1}, respectively.

3.2. Sampling and analysis of the hydrochemistry

Water samples from the springs and ponds were collected once every four hours during autumn and winter and every six hours during summer and spring. Major cation and anion samples were collected in 20 mL acid-washed high-density polyethylene bottles, using 0.45 μm Millipore filters. The cation samples were acidified to pH < 2.0 with concentrated nitric acid to prevent complexation and precipitation. Concentrations of K^+, Na^+, Ca^{2+} and Mg^{2+} were determined with an inductively coupled plasma emission spectrometer (ICP-OES) and Cl^-, NO_3^- and SO_4^{2-} with an ICS-90 ion chromatograph resolving to 0.01 mg L^{-1}. Because of their instability, HCO_3^- concentrations were determined *in situ* by titration with an Aquamerck alkalinity test kit with resolution of 0.05 mmol L^{-1}.

Dissolved organic carbon (DOC) samples were first filtered through 2 mm mesh sieves to remove large particles and aquatic plants debris, and then through 0.2 μm Whatman GF/F filters into brown glass bottles. The bottles were combusted at 450 °C for three hours in a Muffle furnace. To eliminate any biological processes in the bottles, we added 2 mL saturated $HgCl_2$ solution after sampling, then stored the same at 4 °C, and analyzed with an Analytik Jena N/C Multi3100. Particulate organic carbon (POC) was captured on Whatman filters, dried at 65 °C for 48 h and then fumed with concentrated hydrochloric acid in a sealed desiccator for 24 h to remove inorganic carbon. After drying, the

samples were analyzed by Vario MAX CN Elementar with an analytical error < ± 2%.

3.3. Calculation of continuous diurnal HCO_3^- concentration, CO_2 partial pressure and dissolved CO_2 concentration

Ca^{2+} and Mg^{2+} are the major cations, while HCO_3^- is the dominant anion in these waters, and they dominate the electric conductivity (EC) in these aquatic systems. Consequently, continuous concentrations of Ca^{2+}, Mg^{2+} or HCO_3^- can be estimated from the automatically recorded EC data (Liu et al., 2007; Chen et al., 2017). Based on the titrated [HCO_3^-] and the [Ca^{2+}] and [Mg^{2+}] lab analyses, the linear relationships between these ions and EC in the experiments were published by Chen et al. (2017).

Based on the automatic monitoring of diurnal physicochemical parameters by Technology Multiline 350i and major ion determinations by ICP-OES, we employed the geochemical modelling program PHREEQC3 (Parkhurst and Appelo, 1999) to calculate the diurnal pCO_2 in each spring-pond system. This revealed the diurnal pCO_2 changes caused by the C uptake by aquatic ecosystems, and the CO_2 exchanges between air and pond water. Dissolved $CO_{2(aq)}$ values were calculated based on the dominant DIC speciation shift in the inorganic carbon chemistry as a function of pH (Zeebe and Wolf-Gladrow, 2001).

4. Results

4.1. Seasonal and diurnal variability in T, pH and DO of the springs and ponds

All seasonal diurnal changes of T, pH and DO in the spring-pond systems (S-Ps(1) to S-Ps(5)) are summarized in Figs. 3a–e and Tables 1–5 (as Supplementary Data). These trends show that all parameters in the ponds displayed remarkable, but similar seasonal diurnal patterns in variation, with T, DO and pH increasing during daytime and decreasing over the night. P4 had lowest mean pH (7.90–9.60) and diurnal pH range among all ponds. Diurnal changes in DO were more remarkable in October in P3 (3.00 to 20.00 mg/L) and P4 (2.30 to 14.00 mg/L), but less in January (9.10 to 15.30 mg/L and 8.00 to 12.90 mg/l for P3 and P4, respectively). Diurnal changes in the springs, however, were very small or even remained unchanged (Fig. 3, daily variation < 1% in most seasons).

4.2. Seasonal diurnal variabilities of HCO_3^-, $CO_{2(aq)}$, and NO_3^- in the spring-pond systems

The HCO_3^- and $CO_{2(aq)}$ concentrations of springs were relatively stable at the diurnal scale in all seasons (Fig. 3). Generally, spring S4 had the highest HCO_3^- and $CO_{2(aq)}$ concentrations (227–256 mg/L and 5.5–12.4 mg/L, respectively), followed by S5 (164–241 mg/L and 3.5–7.9 mg/L, respectively), S3 (140–240 mg/L and 2.8–5.8 mg/L, respectively) and S2 (121–171 mg/L and 1.1–2.7 mg/L, respectively). The lowest values occurred in S1 (116–159 mg/L and 1.0–2.1 mg/L, respectively). In contrast, concentrations of HCO_3^- and $CO_{2(aq)}$ in the ponds decreased during the daytime, with lowest values occurring at 16:00–18:00 hrs, and increased during the night, with peaks occurring early in the morning (6:00–8:00 hrs): they showed remarkable diurnal changes in all seasons except during the winter (Fig. 3).

Diurnal changes in concentration of nitrate are also shown in Fig. 3. Generally, they are similar to the HCO_3^- and $CO_{2(aq)}$ findings, with nitrate in the ponds decreasing during the day and increasing over the night. The largest amplitude in diurnal changes of NO_3^- in all ponds occurred in the summer and October samplings, and the lowest diurnal changes were in January. Higher NO_3^- concentrations occurred in springs S3 (1.74–35.86 mg/L) and S2 (9.10–20.25 mg/L), and lower in S4 (0.00–0.90 mg/L) and S5 (0.00–0.56 mg/L). Spring S1 showed irregular diurnal NO_3^- changes and fluctuated over the seasons, while S2

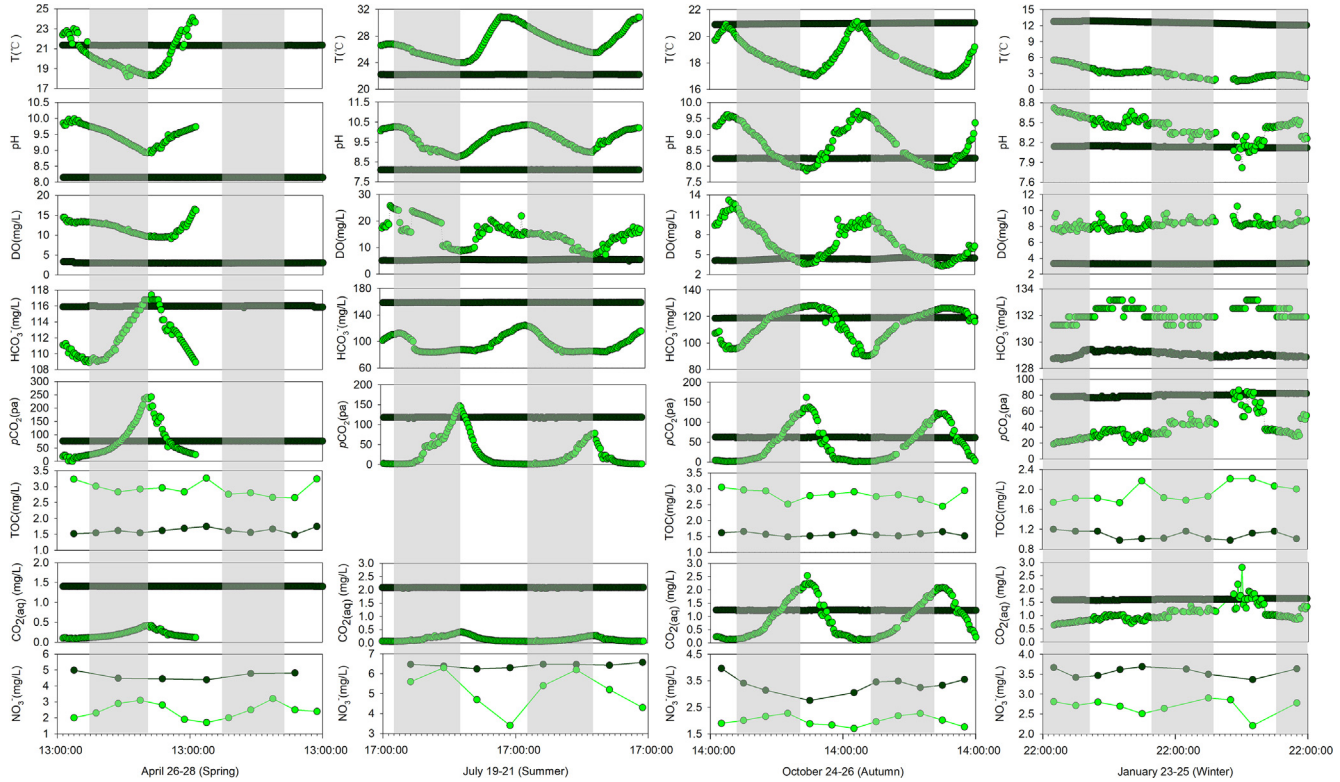

Fig. 3a. Diurnal changes in physiochemical parameters in the experimental bare rock spring-pond system (S-P_S (1)) in four different seasons. Black symbols = springs. Green = ponds. The shaded regions denotes night-time. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

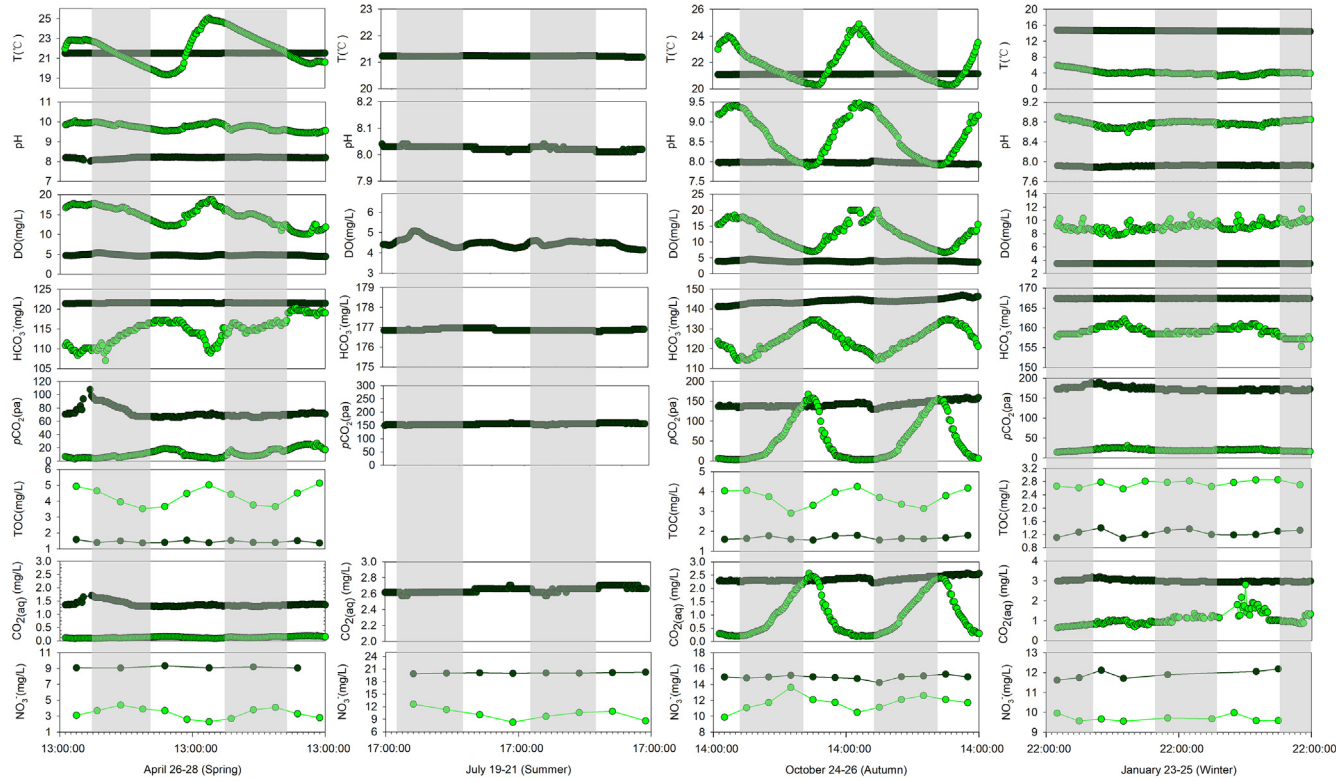

Fig. 3b. Diurnal changes in physiochemical parameters in the experimental bare soil spring-pond system (S-P_S (2)) in the four different seasons. Black = springs. Green = ponds. The shaded regions denote night-time. N.B. Summer physicochemical changes in the pond could not be monitored due to damage to the WTW Technology Multiline 350i instrument. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

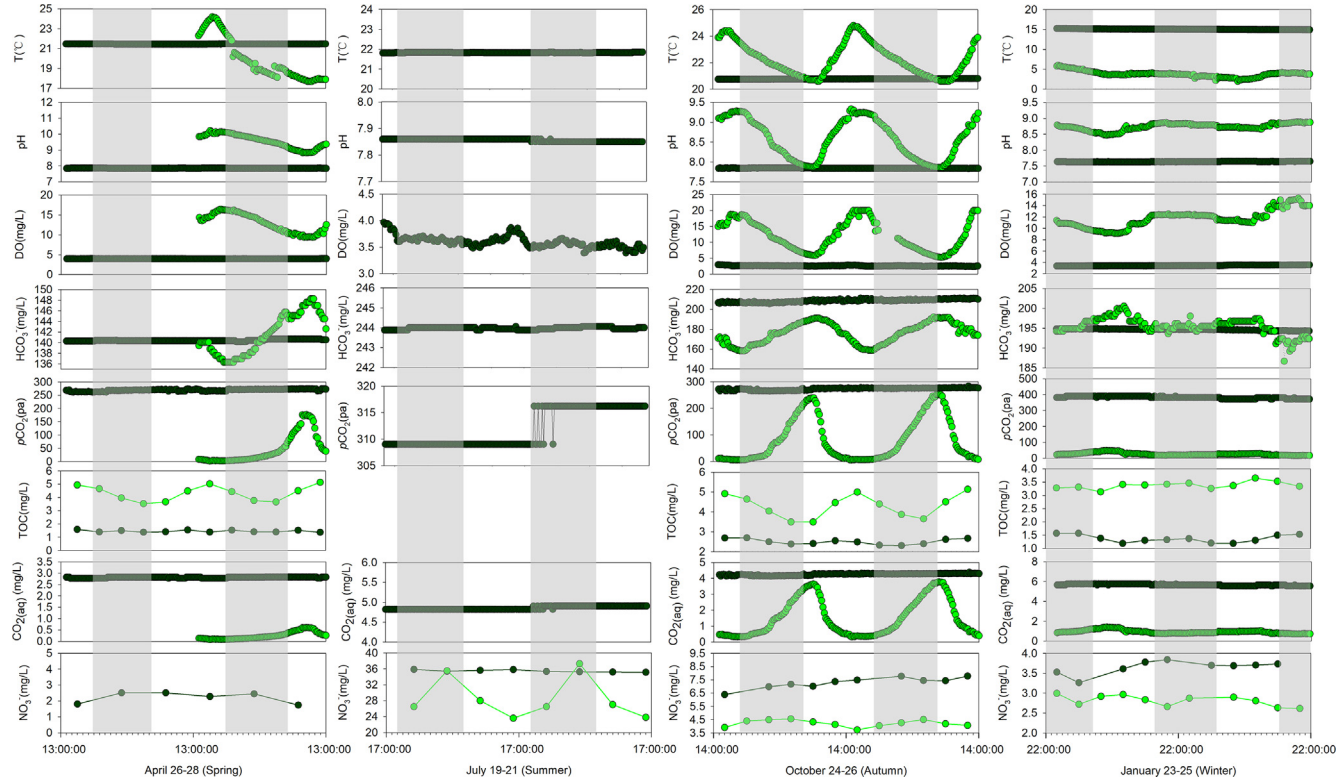

Fig. 3c. Diurnal changes in physiochemical parameters in the experimental cropland spring-pond system (S-P_S (3)) in the four different seasons. Black = springs. Green = ponds. The shaded regions denote night-time. N.B. Summer physicochemical changes in the pond in could not be monitored due to damage to the WTW Technology Multiline 350i instrument. NO_3^- concentration in April is omitted because it was below the limit of detection. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

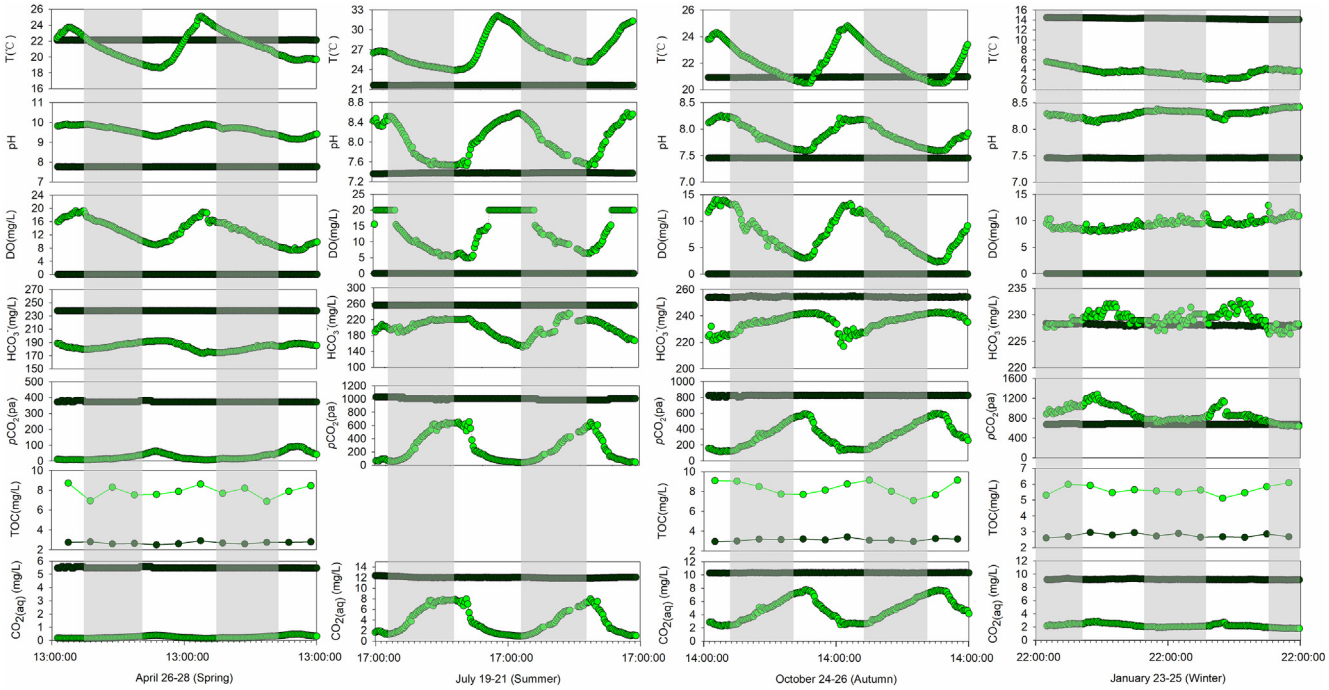

Fig. 3d. Diurnal changes in physiochemical parameters in the experimental grassland spring-pond system (S-P_S (4)) in the four different seasons. Black = springs. Green = ponds. The shaded regions denote night-time. NO_3^- concentrations are omitted because they were below the limit of detection. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and S3 displayed descending NO_3^- concentration, with Summer (July) > Autumn (October) > Winter (January) > Spring (April), demonstrating a marked annual trend. There was a decline in NO_3^- concentration from spring to the pond outlet in all cases.

4.3. Seasonal diurnal variabilities of TOC in the spring-pond systems

As shown in Figs. 3(a)–(e), the diurnal variabilities of TOC in each pond were similar, increasing during daytime but decreasing during night time, while there was little diurnal change for the springs. In addition, the TOC concentrations in each pond were higher than their source spring and showed seasonal differences, i.e., higher during

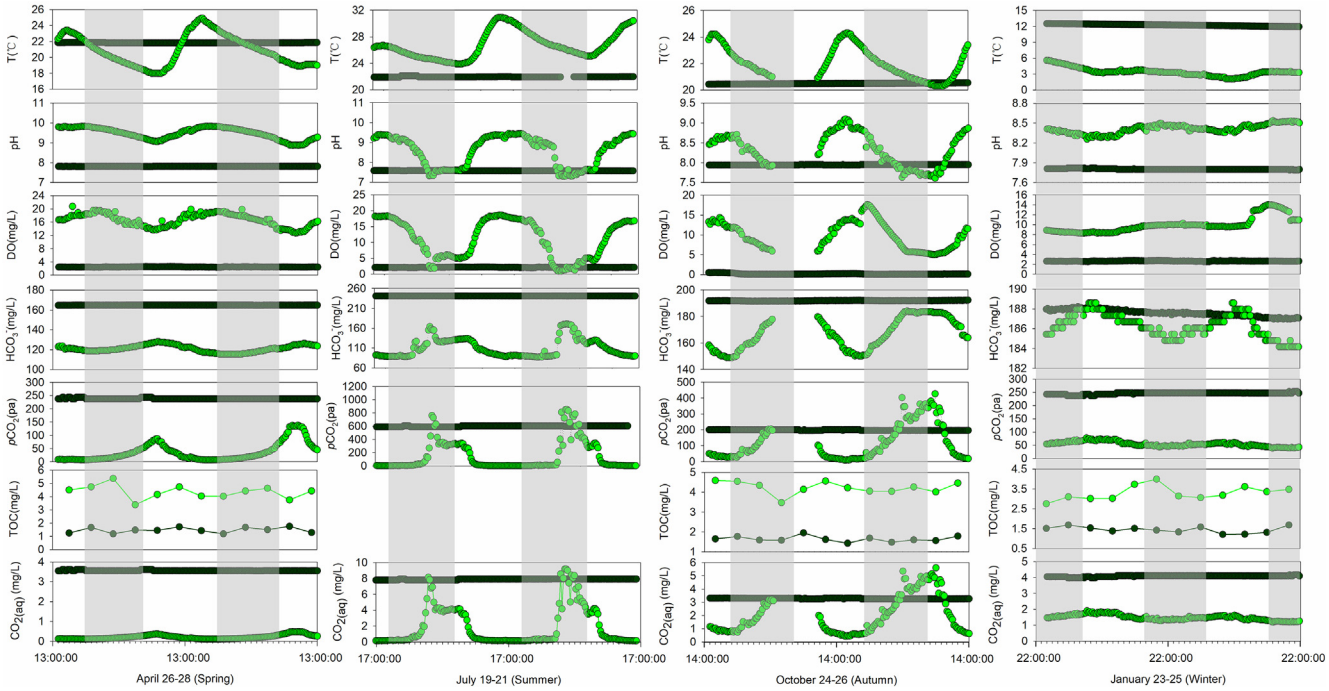

Fig. 3e. Diurnal changes in physiochemical parameters in the experimental shrubland spring-pond system (S-P_S (5)) in the four different seasons. Black = springs. Green = ponds. The shaded regions denotes night-time. NO_3^- concentrations are omitted because they were below the limit of detection. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

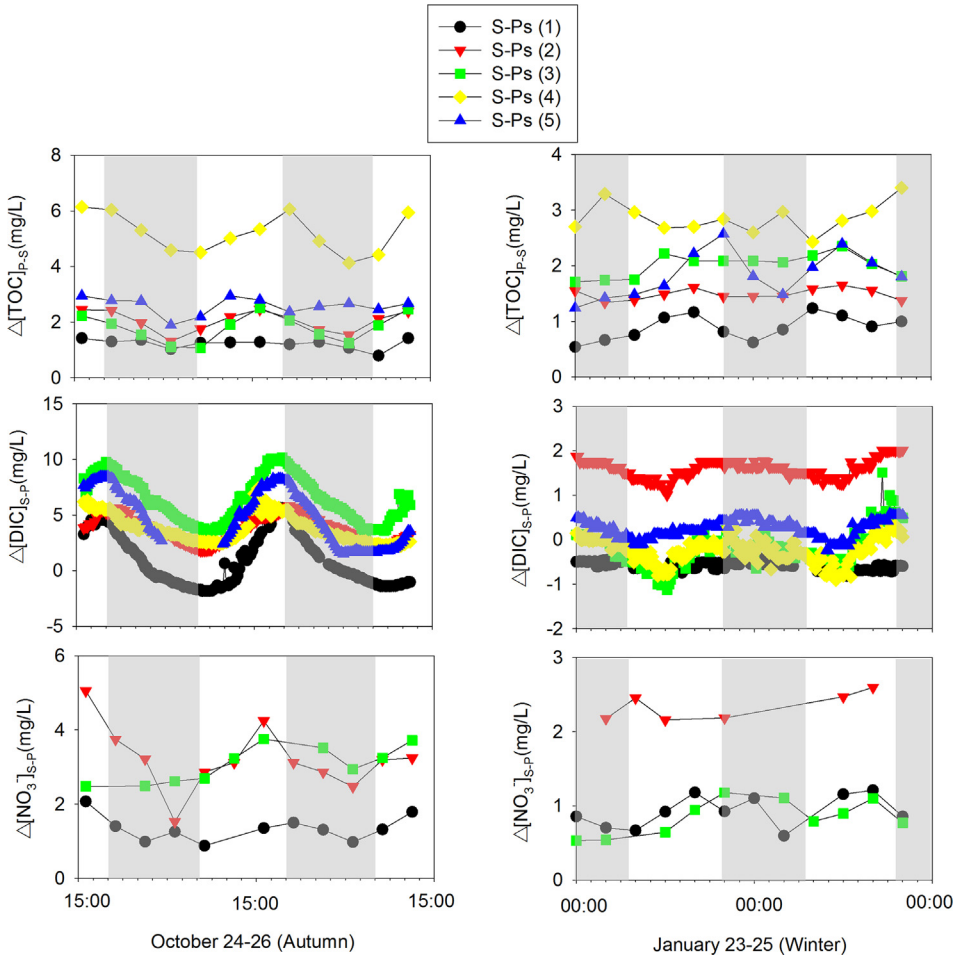

Fig. 4. Diurnal changes in $\triangle[TOC]_{p\text{-}s}$, $\triangle[DIC]_{s\text{-}p}$ and $\triangle[NO_3^-]_{s\text{-}p}$ in spring-pond systems (S-Ps (1) to S-Ps(5)) in autumn and winter. $\Delta[TOC]_{p\text{-}s}$ is net TOC production between the spring and pond, and $\triangle[DIC]_{s\text{-}p}$ and $\Delta[NO_3^-]_{s\text{-}p}$ are net DIC and nitrate loss between spring and pond, respectively. Shaded regions denote night time.

spring and autumn, but lower in winter. S-Ps (4) had the highest TOC concentration in both spring and pond (2.70–3.10 mg/L and 5.60–8.30 mg/L, respectively; Fig. 3), while the values in S-Ps (1) were the lowest (1.10–1.60 mg/L and 1.90–2.90 mg/L, respectively).

The difference in TOC between each spring and its pond was calculated to estimate the net total organic carbon production (△TOC) attributed to aquatic photosynthesis in each pond. The net DIC loss (△DIC) and NO_3^- loss ($\triangle NO_3^-$) in each spring-pond were calculated to investigate their diurnal variations and relationship with △TOC. As shown in Fig. 4, there were prominent diurnal changes of △TOC in autumn, when there was greater biological activity in all pond systems than in winter. Generally, △TOC reached peak values in late afternoon and decreased dramatically during the night-time, with lowest values occurring in the early morning (Fig. 4). Comparing the different land-uses, the highest △TOC occurred in S-Ps (4), simulated grassland, in October and April with a mean value of 5.20 mg/L, and the lowest occurred in S-Ps (1), simulated bare rock, in winter with a mean value of 0.89 mg/L. △DIC also exhibited strong diurnal cyclicity in October, with the maximum values occurring around 18:00 hrs, and minimum values in the early morning. This cyclicity was present but much weaker in winter. Similarly, $\triangle NO_3^-$ showed substantial diurnal changes like those of △TOC in the spring and autumn samplings, but weaker patterns in winter, especially in S-Ps (2), simulated bare soil (Fig. 4).

5. Discussion

5.1. Seasonal variations of DIC, pCO_2 and NO_3^- concentrations in the springs

Seasonal root respiration and organic matter decomposition forced soil CO_2 variation, thus controlling the carbonate weathering rate (Liu et al., 2007; Yang et al, 2012; Zhao et al, 2015; Zeng et al, 2017). The differences in DIC (as expressed by $CO_{2(aq)}$, HCO_3^- and CO_3^{2-}) from the springs can be attributed to the different land-uses (differing soil, type and extent of vegetal cover) in the tanks. Strong respiration and abundant organic matter decomposition in Tank LU4 generated the greatest soil CO_2, leading to the highest DIC concentrations in spring S4 (Fig. 3). In contrast and as expected, the bare rock rubble conditions in Tank LU1 yielded the lowest DIC concentrations because pCO_2 values were lowest there (Zeng et al., 2017). The seasonal variations of DIC and pCO_2 in S2-S5 were large due to the large differences in temperature, soil moisture and solar irradiance levels between the growing and dormant periods.

Like DIC, the NO_3^- in S1-S3 also displayed seasonal variations during the study period, higher in summer/autumn, and lower in winter/spring (Fig. 3). The seasonal difference in nitrate concentration between S1 and S2 was attributed chiefly to the atmospheric nitrogen deposition and biological N fixation in the soils, respectively (Dinnes et al, 2002), the latter controlled by temperature and macrobiotic activity. The highest nitrate concentration, 35.40 mg/L in S3 in summer was due to the use of fertilizer added for corn production.

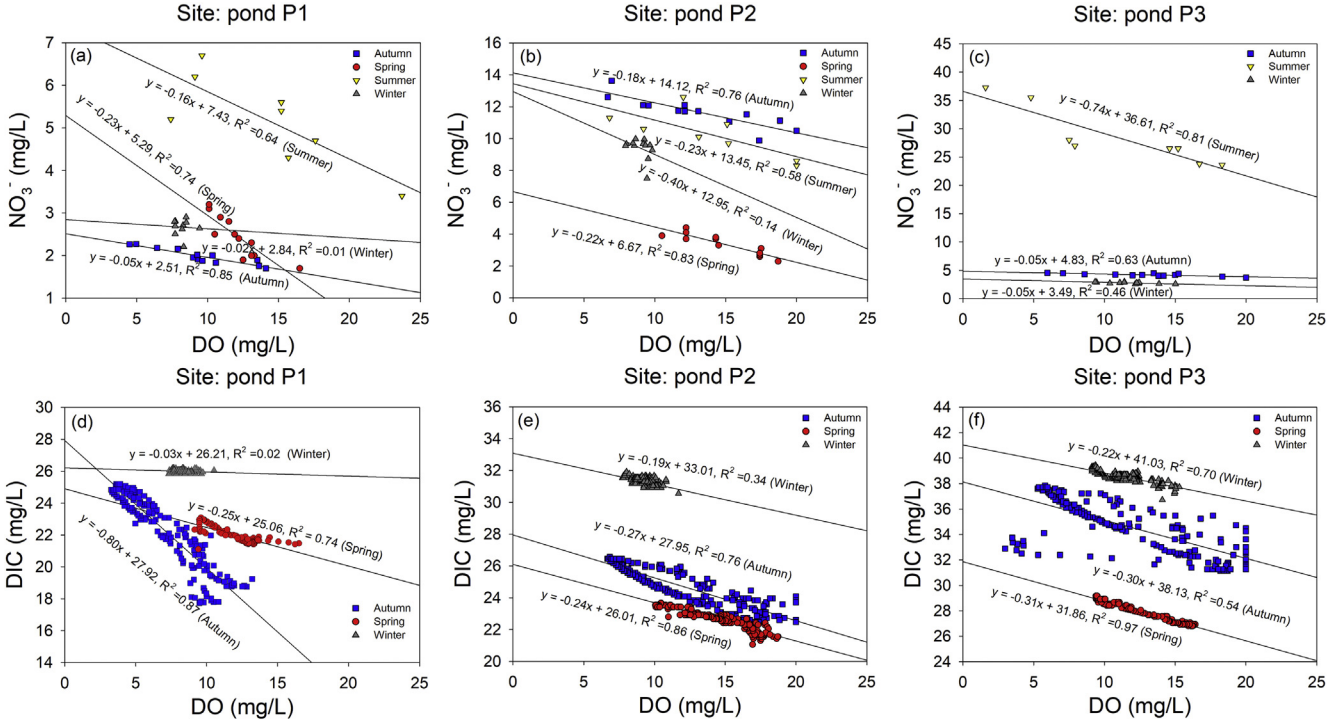
Site: pond P1
Site: pond P2
Site: pond P3
(a)
(b)
(c)
(d)
(e)
(f)
Autumn
Spring
Summer
Winter
NO3- (mg/L)
DIC (mg/L)
DO (mg/L)
y = -0.16x + 7.43, R2 =0.64 (Summer)
y = -0.23x + 5.29, R2 =0.74 (Spring)
y = -0.02x + 2.84, R2 =0.01 (Winter)
y = -0.05x + 2.51, R2 =0.85 (Autumn)
y = -0.18x + 14.12, R2 =0.76 (Autumn)
y = -0.23x + 13.45, R2 =0.58 (Summer)
y = -0.40x + 12.95, R2 =0.14 (Winter)
y = -0.22x + 6.67, R2 =0.83 (Spring)
y = -0.74x + 36.61, R2 =0.81 (Summer)
y = -0.05x + 4.83, R2 =0.63 (Autumn)
y = -0.05x + 3.49, R2 =0.46 (Winter)
y = -0.03x + 26.21, R2 =0.02 (Winter)
y = -0.25x + 25.06, R2 = 0.74 (Spring)
y = -0.80x + 27.92, R2 =0.87 (Autumn)
y = -0.19x + 33.01, R2 =0.34 (Winter)
y = -0.27x + 27.95, R2 =0.76 (Autumn)
y = -0.24x + 26.01, R2 =0.86 (Spring)
y = -0.22x + 41.03, R2 =0.70 (Winter)
y = -0.30x + 38.13, R2 =0.54 (Autumn)
y = -0.31x + 31.86, R2 =0.97 (Spring)

Fig. 5. Relationships between DIC or NO_3^- and DO concentrations in ponds P1 to P3 over the four seasons. All the relationships are significant at $p < 0.01$ except for P1 in winter at $p > 0.1$.

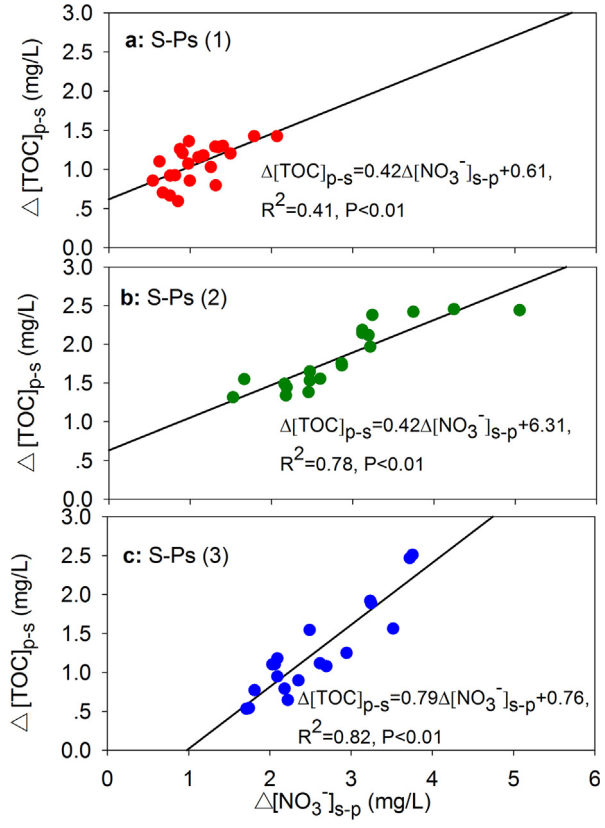

Fig. 6. Plots of $\triangle[TOC]_{p\text{-}s}$ vs $\triangle[NO_3^-]_{s\text{-}p}$ in spring-pond systems S-Ps (1), (2) and (3). $\Delta[TOC]_{p\text{-}s}$ is net TOC production between the spring and pond, and $\Delta[NO_3^-]_{s\text{-}p}$ is net nitrate loss between spring and pond.

5.2. Metabolism controls on the diurnal variations of DIC, NO_3^-, DO and net OC production in the pond ecosystems.

Diurnal variations of DIC, NO_3^- in aquatic ecosystems can be attributed chiefly to aquatic plant metabolism (Chen et al., 2017; Liu et al, 2015; Yang et al, 2015). Intense photosynthesis during the day was accompanied by increased DO production in all ponds. Conversely, respiration of the aquatic plants consumes the DO, with respiration dominating DO variations at night (Chen et al., 2017; Jiang et al, 2013; Yang et al, 2015). As shown in Fig. 3, all of the ponds showed cyclic patterns of increase/decrease in DIC (e.g., HCO_3^- and $CO_{2(aq)}$) and DO over the diurnal cycles, except during winter when biotic activities were presumed at their lowest levels. The pCO_2 variation tracked the DIC variation, also indicating the influence of metabolism. The extremely low pCO_2 measured in late afternoon was consistent with the finding that assimilation of DIC by phytoplankton can draw down pCO_2 to a very low level (=0.1 μatm) and the waters become highly undersaturated with respect to atmospheric CO_2 (Balmer et al, 2011). At the night, pCO_2 in the ponds increased and surpassed even the CO_2 levels in spring waters, reinforcing the presumption of aquatic plant respiration dominating at night.

The NO_3^- uptake rates in aquatic ecosystems have been demonstrated to show seasonal and diurnal variability globally (Simon et al, 2005; Mulholland et al, 2006). As shown in Figs. 3a–c, diurnal cycles in nitrate concentration were found in each pond, during the growing and dormant periods. The lowest values of NO_3^- occurred during the late afternoon, and the highest during the early morning hours. This trend is attributed chiefly to daily biological activities such as assimilation of NO_3^- produced by autotrophic and heterotrophic processes (Mulholland et al, 2006; Heffernan and Cohen, 2010; Rusjan and Mikoš 2010, Jiang et al, 2013), which are highly dependent on water temperature or availability of light (Hessen et al, 1997). A strong negative relationship between the concentrations of DO and NO_3^- were found in P1, P2 and P3 in all seasons except the winter (Fig. 5). Combining all of these results, we conclude that NO_3^- variations in the ponds were caused by uptake during plant photosynthesis, which was followed by

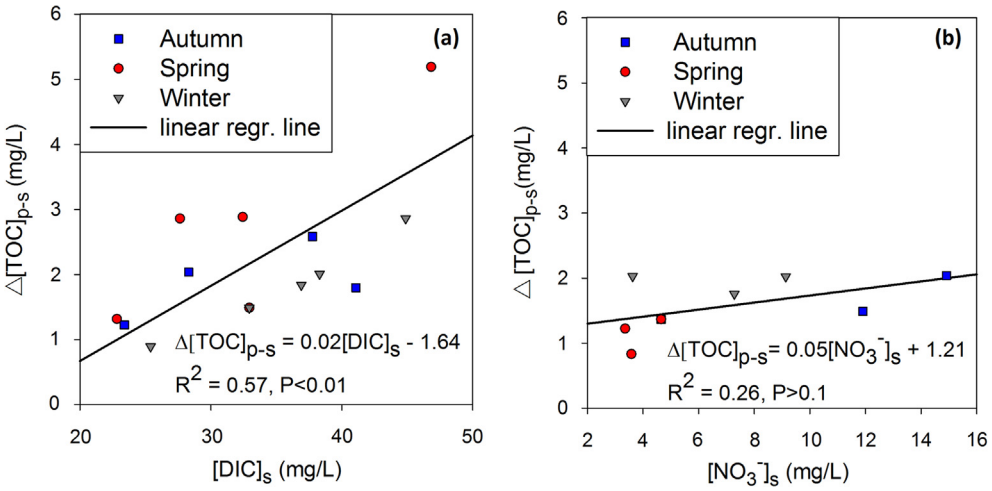

Fig. 7. Relationships between $\triangle TOC_{p\text{-}s}$ and $[HCO_3^-]_s$ ((a), S(1) to S(5)) or $[NO_3^-]_s$ ((b), S(1) to S(3), no data for S(4) and S(5)) of the springs. $\Delta[TOC]_{p\text{-}s}$ is net TOC production between the spring and pond, and $[NO_3^-]_s$ and $[HCO_3^-]_s$ are the nitrate and bicarbonate concentrations of springs, respectively.

the DIC changes shown above.

There were large differences in TOC concentrations between the springs and their ponds (Fig. 3), indicating substantial OC production by aquatic photosynthesis in the pond ecosystems. The net OC production ($\triangle[TOC]_{P\text{-}S} > 0$) between each spring and pond was matched by DIC and NO_3^- losses between them, as shown in Fig. 3, as well as the negative relationship between NO_3^- and DO (Fig. 5). Both ΔNO_3^- and ΔDIC in S-Ps (1) to S-Ps (3) showed seasonal patterns and diurnal cycles that closely matched △TOC, especially in the autumn growing season when aquatic photosynthesis was most active (Fig. 4). Fig. 6 further shows the significant positive relationship between OC production and net NO_3^- uptake in S-Ps (1) to S-Ps (3).

5.3. Carbon limitations on aquatic ecosystem primary production

Intense photosynthesis in aquatic ecosystems can raise the pH by consuming dissolved CO_2 (Talling, 1976). However, ecosystem respiration will increase CO_2 concentrations at night as well, decreasing the pH and potentially driving calcite dissolution which will increase DIC concentration (Cicerone et al., 1999; Liu et al., 2008). The DIC in our ponds was actively assimilated by submerged plants during the day, when temperature and solar irradiance levels increased. The pH in the ponds showed substantial diurnal change, with a range of 7.50–10.00 during the period of study, as illustrated in Fig. 3. DIC in this pH range was dominated by HCO_3^-, with extremely low amounts of $CO_{2\ (aq)}$ (< 10%, Fig. 1). It is well known that $CO_{2\ (aq)}$ is the favored substrate for aquatic plant photosynthesis. The elevated pH may reduce plant growth and photosynthesis by limiting $CO_{2(aq)}$, the effect known as 'carbon limitation' (Chen et al., 1994; Riebesell et al., 1993; Van Dam et al., 2018; Verspagen et al., 2014). When $CO_{2\ (aq)}$ is low, some algae can use HCO_3^- as their carbon source for primary production (Price et al., 2011). However, other algae can use only dissolved CO_2 (Riebesell et al., 1993) for photosynthesis because utilization of bicarbonate requires a specific bicarbonate uptake system (Tortell, 2000; Visser et al., 2016). On the other hand, as a fundamental nutrient for primary production, nitrate is also related to the OC production in freshwater ecosystems. The higher concentrations of NO_3^- found in streams in agriculturally exploited catchments stimulates the NO_3^- uptake by aquatic ecosystems (Mulholland et al., 2006), and thus accumulation of OC in water (Heffernan and Cohen, 2010) and/or sediments (Huang et al., 2018).

The production of organic matter is generally accompanied by prominent deviations of C, N and P in the ratio 106:16:1 (known as Redfield Ratio) for the elemental stoichiometry of marine organic matter (Redfield et al., 1963). Compared to C, N and P were for a long while regarded as the dominant limiting nutrients for aquatic ecosystems and eutrophication (Howarth and Marino, 2016; Schindler et al., 2016). However, several studies have shown that elevated CO_2 uptake can increase the carbon:nutrient stoichiometry of phytoplankton (Riebesell et al., 2007; Verschoor et al., 2013; Verspagen et al., 2014). The rising CO_2 levels can increase phytoplankton C:N stoichiometry, the effect being more prominent at low than at high nutrient load. Furthermore, even under severe nutrient-limited conditions, primary producers still have the capacity for photosynthesis when sufficient light and DIC are available (Verschoor et al, 2013). Our results show that in S-Ps (1) to S-Ps (3), where DIC concentrations are S3 > S2 > S1, the C:N ratios (i.e., the slopes in Fig. 6) increased with DIC, i.e., higher DIC input resulted in higher values in C:N stoichiometry.

The NO_3^- and PO_4^{3-} in S-Ps (4) and S-Ps (5) were extremely low (Chen et al., 2017), and the supply of DIC in these waters exceeded NO_3 and PO_4^{3-}, possibly by several orders of magnitude. However, the △TOC in S-Ps(4) and S-Ps(5) were even higher than those in the S-Ps (1,2,3) systems, which had greater NO_3^- supply. Therefore, the carbon limitation may indicate that the photoautotrophs (dominated by *Charophyta* and *Spirogyra*) in karst surface aquatic ecosystems cannot use the total DIC for photosynthesis, but only the dissolved CO_2, which comprises < 1% of total DIC at pH > 8 (characteristic of karst surface aquatic ecosystems). The DIC input gradient caused by the different land uses in our experiments indicated that greater DIC input alleviated the C limitation by supplying more CO_2 for aquatic plant photosynthesis in the waters. In Fig. 7 we plot the relationship between △TOC and DIC ((a), S-Ps (1) to S-Ps(5)) or NO_3^- ((b), S-Ps (1) to S-Ps(3)) inputs in October, January and April to detect different impacts of C and N input on net OC production. DIC inputs show a more significant positive relationship with the net OC production than do NO_3^- inputs. Therefore, it is suggested that in karst terrains surface waters with high pH values, carbon (or DIC) input may become the dominant factor that limits aquatic primary production.

5.4. Implication for eutrophication control and carbon sink projection experiencing land-use and climate changes

The above finding that carbon can limit aquatic primary production may have two implications: one for control of eutrophication rates and the other for carbon sink rates where there is land-use and/or climate change.

(1) Implications for eutrophication control

Whole-lake experiments by Schindler and others since 1971 have shown conclusively that phosphorus is the major cause of eutrophication in many freshwater lakes (Schindler et al., 2008, 2016). However,

contrary to this phosphorus (P)-only paradigm, control of nitrogen (N) input is widely adopted to alleviate eutrophication in New Zealand (Abell et al., 2010) and many other countries (Seitzinger et al., 2006; Jarvie et al., 2018). A review of published results of nutrient enrichment experiments in lakes showed that N more frequently limited lake productivity than P (Abell et al., 2010), indicating the complexity in eutrophication controls. In fact, about 20 years ago, Hein (1997) found that photosynthesis by phytoplankton in lakes with low to intermediate DIC concentrations was seriously restricted when CO_2 concentrations declined. In particular, photosynthesis was limited by low CO_2 concentrations during phytoplankton blooms in hardwater alkaline lakes. Our results here further confirm this. Therefore, eutrophication control in freshwater lakes may be limited not only by P and N, but also by C.

(2) Implication for carbon sink projection with land-use and global climate change

Terrestrial and aquatic primary production are closely related to the local land use activity. A growing number of studies have found that rising atmospheric CO_2 levels and increasing agricultural land-use can enhance the carbonate weathering rates, leading to increasing input of DIC to surface water systems (Andrews and Schlesinger, 2001; Macpherson et al., 2008; Raymond et al., 2008). Combined with our findings on carbon limitation, it is projected that the elevation of DIC concentrations will enhance primary production in surface-water ecosystems, and thus increase the OC production, implying that there will be an increase in the net carbon sink under land-use and global climate changes, showing long-term implications of this process (biological carbon pumping) in affecting the water chemistry, eutrophication and other issues in karst terrains.

6. Conclusions

In this study, we documented seasonal and diurnal variations in DIC, NO_3^-, TOC and related hydrochemical parameters (pH and DO) in model spring-pond ecosystems with different land-use activities at the Shawan Karst Test Site, SW China. It was found that diurnal changes in DIC, NO_3^- and TOC concentrations in the ponds were determined by aquatic ecosystem metabolism. The bare rock (LU1) and bare soil (LU2) experiments produced very low DIC and NO_3^- additions to their downstream spring-pond ecosystems (S-Ps (1) and S-Ps (2)), resulting in lower OC production in the latter. The cropland (LU1) yielded higher DIC and NO_3^- to its pond (P3) due to growth of the corn and use of fertilizer, which enhanced the OC production in the pond ecosystem. Highest productivity and best vegetation cover in the grassland (LU4) and shrubland (LU5) experiments (with higher N retention in soils) resulted in highest DIC but only limited NO_3^- addition to the aquatic ecosystems (P4 and P5). The highest DIC concentration in the grassland resulted in the largest OC production among the ponds, even though the NO_3^- addition was smaller there. These results indicate that OC production in the ponds with elevated pH was limited by DIC fertilization, i.e., rising DIC supply counters the carbon limitation, indicating that the photoautotrophs (dominated by *Charophyta* and *Spirogyra*) in karst ecosystems cannot use the total DIC, but only the dissolved CO_2 component (< 1% of total DIC where pH > 8.2). This may have implications for control of eutrophication in karst aquatic ecosystems, i.e., rates of eutrophication in freshwater ecosystems (rivers, lakes and reservoirs) may be regulated not only by N and/or P but also by C. It is also projected that there will be an increase in OC sequestration with the current land-use and global climate change-driven increases in DIC, due to carbon limitation of aquatic primary production.

Declaration of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhydrol.2019.04.090.

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